Antiviral activity of glycyrrhizic acid[1]

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Summary. Glycyrrhizic acid inhibits the growth of several DNA and RNA viruses in cell cultures and inactivates Herpes simplex 1 virus irreversibly.

A triterpenic component of *Glycyrrhizia glabra* roots, glycyrrhizic acid, has been found endowed with antiviral action. At concentrations well tolerated by uninfected cells, glycyrrhizic acid inhibits both growth and cytopathic effect of vaccinia (VV), herpes simplex (HSV), Newcastle disease (NDV) and vesicular stomatitis (VSV) viruses while being ineffective on poliovirus 1 (PV). At the same concentrations, glycyrrhizic acid is also able to inactivate HSV irreversibly.

Materials and methods. Human aneuploid HEp2 cells (ATCC, Rockville) and virus strains were the same as those already used in previous studies[2,3]. Glycyrrhizic acid (ammonium salt) was furnished by Fluka (Switzerland). The experiments were carried out on 16-h-old cell monolayers which were maintained in Eagle's minimum essential medium containing 2% calf serum and adjusted to pH 7.4 (MEM).

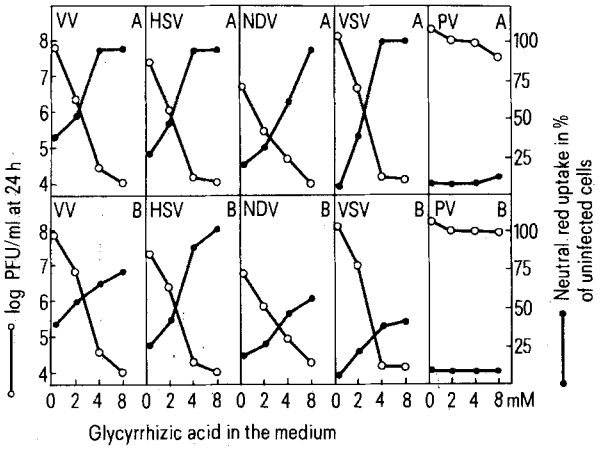

Fig. 1. Effect of different concentrations of glycyrrhizic acid, added to cell cultures soon after (A) or 4 h after (B) infection, on virus growth and cytopathology.

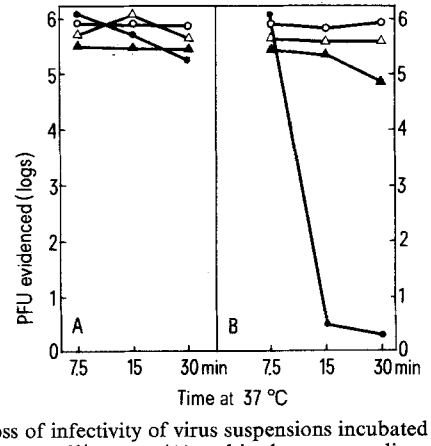

Fig. 2. Loss of infectivity of virus suspensions incubated at 37 °C in drug-free MEM 2% serum (A) and in the same medium containing 8 mM glycyrrhizic acid (B). Empty circles: VV; full circles: HSV; empty triangles: NDV; full triangles: VSV.

The maximum non-cytotoxic concentration (MNCTC) of glycyrrhizic acid was determined in cell cultures (3×10^7 cells per sample) incubated at 37° for 48 h. Cell damages were evaluated by microscopic observation of Giemsa stained cells and by measuring the intracellular uptake of neutral red (100 µg/ml of drug-free medium for 1 h at 37 °C) at 530 nm[4]. The inhibitory effect of glycyrrhizic acid on virus growth was tested on cell cultures in stationary tubes (2×10^6 cells per tube, 3 tubes per sample) which were infected with 5 plaque forming units (PFU) per cell at 20 °C for 1 h, washed 3 times in MEM and incubated at 37 °C in the same medium. Glycyrrhizic acid was added to infected cultures soon after incubation at 37 °C, or later on (see results). Cytopathic effects (CPE) were evaluated after 24 h at 37 °C by the same techniques as those referred to above for cytotoxicity. Infectious virus yield was measured by the agar method of Dulbecco and Vogt[5] starting from whole infected culture samples which were frozen and thawed (−30 °C and +20 °C twice and freed of cell debris by centrifugation at 3000 rpm for 5 min. In the case of VV and HSV the agar medium was added to cell monolayers 30 h after infection. By this method, errors ranging from 23% (for PV) to 36% (for NDV) were found.

Results. Data in figure 1 show that glycyrrhizic acid is endowed with a strong inhibitory activity on growth of VV, HSV, NDV and VSV, when added to infected cultures at 8 mM and 4 mM, that is at ½ and ¼ of the MNCTC. Drug treatments starting at time 0 after infection not only inhibit virus growth, but also prevent CPE completely, so that no difference can be found, at microscope examination, between drug treated infected cells, and uninfected controls. Drug treatments starting 4 h after infection when synthesis of virus macromolecules is in progress, still inhibit virus growth completely, while only reducing the extent of CPE. Under the same conditions, glycyrrhizic acid is ineffective on both growth and cytopathology of PV.

In addition, glycyrrhizic acid is also able to produce irreversible inactivation on HSV particles HSV suspensions lose more than 5 logs of infectivity if incubated at 37 °C for 15 min in the presence of a drug concentration which is well tolerated by uninfected cell for more than 48 h. In contrast VV, NDV and VSV are not or only slightly affected. (figure 2).

Research is in progress to define mode and mechanism of antiviral activity of glycyrrhizic acid. The results shown suggest that glycyrrhizic acid interacts with virus structures (conceivably proteins) producing different effects according to the viral stage affected: inactivation of free virus particles extracellularly (besides HSV, also VV, VSV and NDV might be inactivated, although reversibly); prevention of intracellular uncoating of infecting particles; impairment of the assembling ability of virus structural components.

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